



## Anatomy, palynology and seed micromorphology of Turkish rare *Verbascum racemiferum* Boiss. & Hausskkn Ex Boiss. (Scrophulariaceae)

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### Abstract

*Verbascum racemiferum* Boiss. & Hausskkn ex Boiss. is a member of the *Verbascum* L. (Scrophulariaceae) genus called as "Sığırkuyruğu" in Turkish name. It is a rare species that grows in Turkey which is the center of endemism for *Verbascum* species. In this study, the anatomical, palynological and seed micromorphological features of *V. racemiferum*, which is known to grow only in Mardin in the Southeastern Anatolia region, were investigated for the first time. In the root cross-section, the xylem elements occupy a considerable area. In the stem cross-section, the upper part of the epidermis cells is surrounded by a separate cuticle layer and the pith region covers a large area. The main vein of the leaf is shaped as collateral bundle. Idioblasts were observed in the leaves of *V. racemiferum*. Pollen grains of *V. racemiferum* are tricolporate-tricolpate, spheroidal; P/E ratio is 0.96 and exine ornamentation is reticulate. Seeds of *V. racemiferum* are brown and oblong-ovate to prismatic and alveolate. The apex is acute beak. The seed coat ornamentation is irregular polygonal cells, with densely and distinct vesicles. In addition, this study will be a reference to studies on the genus *Verbascum*.

**Key words:** anatomy, Mardin, pollen, seed, SEM, *Verbascum*

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### Türkiye nadir *Verbascum racemiferum* Boiss. & Hausskkn Ex Boiss. (Scrophulariaceae) anatomisi, palinolojisi ve tohum mikromorfolojisi

#### Özet

*Verbascum racemiferum* Boiss. & Hausskkn ex Boiss. "Sığırkuyruğu" olarak bilinen *Verbascum* L. (Scrophulariaceae) cinsinin bir üyesidir. Tür, *Verbascum* türleri için endemizm merkezi olan Türkiye'de yetişen nadir bir türdür. Bu çalışmada Güneydoğu Anadolu bölgesinde sadece Mardin'de yetiştiği bilinen *V. racemiferum*' un anatomik, palinolojik ve tohum mikromorfolojik özellikleri ilk kez araştırılmıştır. Kök kesitinde ksilem elemanları hatırı sayılır bir alan kaplar. Gövde kesitinde epidermis hücrelerinin üst kısmı ayrı bir kütikül tabakası ile çevrelenmiş olup, öz bölgesi geniş bir alanı kaplamaktadır. Yaprığın ana damarı kollateral demet şeklindedir. *V. racemiferum*' un yapraklarında idioblastlar gözlemlendi. *V. racemiferum*' un polen taneleri trikolporat-trikolpat, küreseldir; P/E oranı 0,96 olup ekzin süslemesi ağ şeklindedir. *V. racemiferum*' un tohumları kahverengidir ve dikdörtgen-oval ila prizmatik ve alveolattır. Tepe noktası keskin gagadır. Tohum kabuğu süslemesi, yoğun ve belirgin keseciklere sahip düzensiz çokgen hücrelerdir. Ayrıca bu çalışma *Verbascum* cinsi üzerinde yapılan çalışmalara da referans olacaktır.

**Anahtar kelimeler:** anatomi, Mardin, polen, SEM, tohum, *Verbascum*

#### 1. Introduction

*Verbascum* L. genus, which is also known as "Sığırkuyruğu" in Anatolia, is one of the largest genera of the Scrophulariaceae family [1]. The genus, which is represented by more than 360 species in the world, grows widely in the temperate regions of the Northern Hemisphere, especially in the eastern part of Eurasia [2]. The general distribution area of *Verbascum* species is Anatolia, predominantly the Irano-Turanian phytogeographic region [3]. In Turkey, it is

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distributed with a total of 257 species and 132 additional hybrids under 13 groups, 80% of which are endemic and 130 of which are hybrids [3-4].

The genus *Verbascum* is one of the largest genera in terms of the number of species it contains, which is known to have problems in its diagnosis and taxonomy in general. There are few studies on the morphological and anatomical features of the genus [5]. There are Scanning Electron Microscope (SEM) studies on the pollen morphology of *Verbascum* species [6-13]. Additionally, researchers conducted studies on the seed micromorphology of the *Verbascum* genus [7, 13-16]. Anatomical studies on the genus are few [11-12, 17-20], there are still deficiencies and unstudied taxa in Turkey.

*Verbascum racemiferum* is a rare species that grows in Mardin in the Irano-Turanian phytogeographic region and is rare in our country [3]. According to Davis's grid system, *V. racemiferum* spreads in the C8 square.

In this study, anatomy, pollen, and seed micromorphology features of the rare *V. racemiferum* species were investigated for the first time. In addition, this study will be a reference to studies on the genus *Verbascum*.

## 2. Material and methods

This study was conducted on the rare *Verbascum racemiferum* species collected from Artuklu, Derik and Kızıltepe districts of Mardin province between 2022 and 2023 (Table 1). The plant collection studies were carried out in May, which is the flowering period of the *Verbascum* species. The treatises of Davis et al. [3] and Karavelioğulları [4] were used to identify the collected samples.

Collected specimens were preserved in falcon tubes in 70% alcohol for use in anatomical studies. Sections taken from the root, stem and leaf parts of the plants with the help of a razor were prepared by staining with safranin-fast green and examined under the light microscope and photographed [21].

Pollen samples used for palynological examinations were made according to Erdtman [22]. The pollen preparations examined were prepared according to the method of Wodehouse [23]. Anthers taken from the flowers of the herbarium specimens were placed on a clean slide and the thecae were opened with the help of a needle. It was cleaned by dropping a few drops of 96% ethanol onto it. This process was repeated twice and the alcohol was evaporated. The coverslip was covered by adding a few drops of glycerin gelatin dye melted on the heater and examined under a light microscope. For SEM, pollens were mounted directly onto the stubs using double-sided adhesive tape and coated with gold. The photomicrographs were taken with a ZEISS EVO 50 scanning electron microscope. The values of P (polar axis length), E (equatorial diameter), Clg (Colpus longitude [length]), Clt (Colpus latitude [width]), Plg (Polar longitude [length]), Plt (Polar latitude [width]), Ex (Exine thickness), and In (Intine thickness) were measured, and the P/E ratio was calculated, Apt (Aperture type), and Or (Ornamentation) for 30 pollen grains were measured under light microscope. The terminology of the pollen follows that of Punt et al. [24]. The values are presented as minimum, maximum and mean, that is represented in Table 3.

The measurements of the seeds were primarily made macromorphologically (length, width etc). Thirty mature seeds belonging to the *V. racemiferum* species were measured and their average values were taken. For SEM, seed residues were removed by distilled water treatment, air-dried seeds were then mounted on the cobs and covered. Photomicrographs were taken with a ZEISS EVO 50 scanning electron microscope. Terminology for the description of morphological features of mericarps has been applied [7, 14, 16]. SEM photographs of the species were interpreted.



Figure 1. General appearance of *V. racemiferum*

Table 1. *V. racemiferum* species used for anatomy and morphology studies and collected localities

Species	Group	Collection areas and habitat	Collector	Collector's number
<i>V. racemiferum</i>	F	Mardin: Kızıltepe, Ğurs villages, Karaman village location, roadside, rocky slope, 37°16'26"N 40°38'41"E, 620 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 249 M.Kılıç 320
		Mardin: Kızıltepe, Ğurs villages, after Karaman village, winding roadside, rocky slope, 37°16'40"N 40°38'24"E, 637 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 250 M.Kılıç 321 M.Kılıç 376
		Mardin: Kızıltepe, Başdeğirmen road, roadside, rocky slope, 37°16'31"N 40°32'41"E, 655 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 323-2 M.Kılıç 324
		Mardin: Artuklu, Yenişehir Neighborhood, Turkmenler road location, roadside, rocky slope, 37°18'36"N 40°42'46"E, 831 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 251
		Mardin: Artuklu, Yenişehir Neighborhood, Turkmenler road location, roadside, rocky slope, 37°18'38"N 40°42'46"E, 822 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 255 M.Kılıç 369
		Mardin: Derik, district exit location, roadside, stony area, 37°22'30"N 40°16'33"E, 862 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 276
		Mardin: Derik, Derik-Mazıdağı road, 2-3 km from Derik, roadside, rocky slopes, 37°23'20"N 40°17'19"E, 1.004 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 277
		Mardin: Derik, Derik-Mazıdağı road, 5-7 km from Derik, roadside, rocky slopes, 37°23'52"N 40°17'49"E, 1.083 m.	M. Kılıç F. Mungan Kılıç	M.Kılıç 278

### 3. Results

In this study, various features of anatomy, pollen, and seed structure of rare *Verbascum racemiferum* species are stated. Biometric measurements of the root, stem, and leaf tissues and cells are given in Table 2 and shown in Figure 2, 3, 4. The characteristics of pollen grains are summarized in Table 3 and shown in Figure 5. The morphological characters of the seed grains, including their size, shape, color, and surface characteristics, are summarized in Table 4 and shown in Figure 6.

#### 3.1. Anatomy

The root is of this species, the outer protective tissue peridermis usually consists of 7-10 layered cells. The primary parenchyma, located under the peridermis, comprised of oval, quadrangular, irregular cell rows limited to a narrow area. There is a multilayered secondary parenchyma between the primary parenchyma and the vascular bundle. There are 3-5 rows of phloem cells under the parenchyma. The cambium is indistinct. Xylem covers a larger area and fills the middle of the root. Tracheal cells are irregularly located, larger than tracheid cells, and longer than they are wide. Phloem occupies a narrower area than xylem (Figure 2, Table 2).

When cross-sections taken from the stem of *V. racemiferum* are examined; The epiderma consists of a single layer of regular cells and well-arranged cells. There are multicellular, glandular and non-glandular hairs in the epidermis. There are 3-5 rows of collenchyma cells just below the epidermis. After the collenchyma layer comes the parenchyma, which is generally wider than it is long and consists of oval and quadrangular cells. There are 2-4 rows of sclerenchyma layers under the parenchyma. Xylem covers a larger area than phloem. The trachea is usually longer than they are wide. The core region of the stem consists of large and small parenchymatic polygonal and round shaped cells (Figure 3, Table 2).

In the cross-section of the leaves of the species, the outer surface of the adaxial and abaxial epidermis is surrounded by a 2.25-6.21  $\mu$  thick cuticle. The adaxial and abaxial epidermis are single-layered and often have amaryllis-type stomata surrounded by 3-4 epidermis cells. The mesophyll consists of 2-3 rows of palisade parenchyma cells under the upper epidermis and 1-2 rows above the lower epidermis. Between the lower and upper palisade parenchyma cells, there are sponge parenchyma cells consisting of 2-3 rows of cells. There are glandular, eglandular and multicellular branched hairs on the adaxial and abaxial epidermis surfaces. Vasculer bundles are collateral. It is well developed and has a crescent shape that curves inwards. Xylem is located towards the upper epidermis, and phloem is located towards

the lower epidermis. In the xylem, tracheal elements are arranged radially, and there are thin-walled parenchymatic cells between them. Phloem lies below the xylem. There are 8-10 rows of distinct parenchyma cells under the upper epidermis. There are 15-20 rows of parenchymatic tissue under the phloem, down to the lower epidermis. The midrib is well developed. The parenchyma layer surrounding the vascular bundle covers a large area. Parenchymal tissue cells are polygonal and tightly arranged. Additionally, idioblasts are observed in the mesophyll tissue of the leaves (Figure 4, Table 2).

Table 2. The anatomical measurements of *V. racemiferum*

Tissues	Width ( $\mu$ )			Length( $\mu$ )		
	Min.	Mak.	Mean $\pm$ S.	Min.	Mak.	Mean $\pm$ S.
<b>Root</b>						
Peridermis cell	7.27	47.63	25.38 $\pm$ 10.25	6.37	22.48	16.50 $\pm$ 4.72
Parenchyma cell	9.70	53.65	28.60 $\pm$ 11.17	11.17	26.26	17.74 $\pm$ 5.12
Phloem cell	2.80	12.54	6.53 $\pm$ 3.01	2.32	7.59	4.48 $\pm$ 1.68
Trachea cell	14.53	81.49	44.51 $\pm$ 19.72	15.27	104.10	50.70 $\pm$ 27.13
<b>Stem</b>						
Cuticle	-	-	-	3.53	9.02	6.83 $\pm$ 1.88
Epidermis cell	11.93	31.68	19.80 $\pm$ 4.75	8.66	20.94	13.22 $\pm$ 3.10
Collenchyma cell	11.47	25.60	17.08 $\pm$ 3.78	9.54	21.74	15.20 $\pm$ 3.46
Parenchyma cell	12.85	41.66	23.85 $\pm$ 7.92	10.42	34.12	16.54 $\pm$ 5.34
Phloem cell	3.53	12.56	8.71 $\pm$ 2.80	3.62	10.15	6.31 $\pm$ 1.93
Trachea cell	8.88	18.09	12.65 $\pm$ 2.55	7.50	19.06	13.30 $\pm$ 3.80
Pith cell	49.85	130.29	83.22 $\pm$ 24.36	50.51	137.25	87.36 $\pm$ 28.76
<b>Leaf</b>						
Cuticle	-	-	-	2.25	6.21	3.88 $\pm$ 1.00
Upper epidermis cell	9.09	36.09	23.25 $\pm$ 10.20	8.68	29.09	15.85 $\pm$ 6.12
Palisade parenchyma	9.60	18.46	13.81 $\pm$ 2.34	26.72	48.48	33.20 $\pm$ 6.34
Spongy parenchyma	10.48	27.68	17.37 $\pm$ 3.63	12.72	24.98	17.77 $\pm$ 3.70
Mesophyll layer	-	-	-	114.71	249.51	199.07 $\pm$ 38.25
Lower epidermis cell	4.09	36.66	17.73 $\pm$ 8.75	6.11	23.02	12.30 $\pm$ 4.50

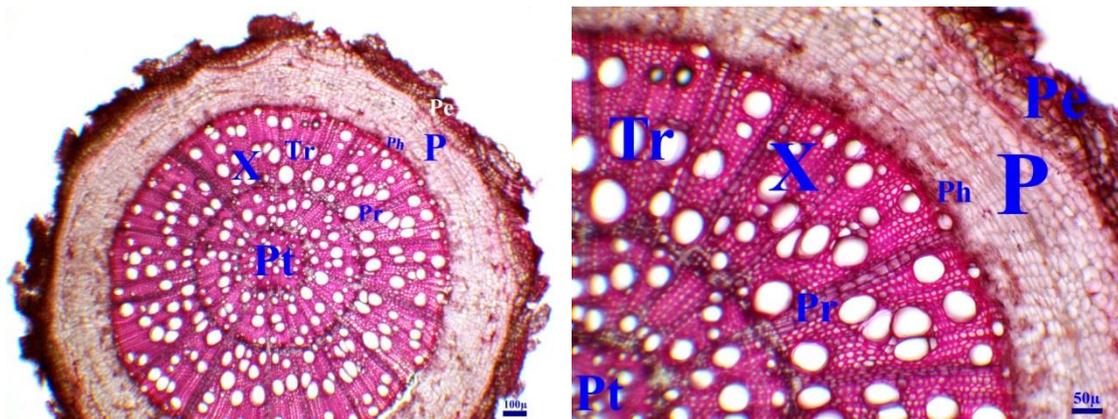


Figure 2. Cross-section of the root of *V. racemiferum*. Pe: Periderm, P: Parenchyma, Ph: Phloem, X: Xylem, Pr: Pith ray, Tr: Trachea, Pt: Pith region

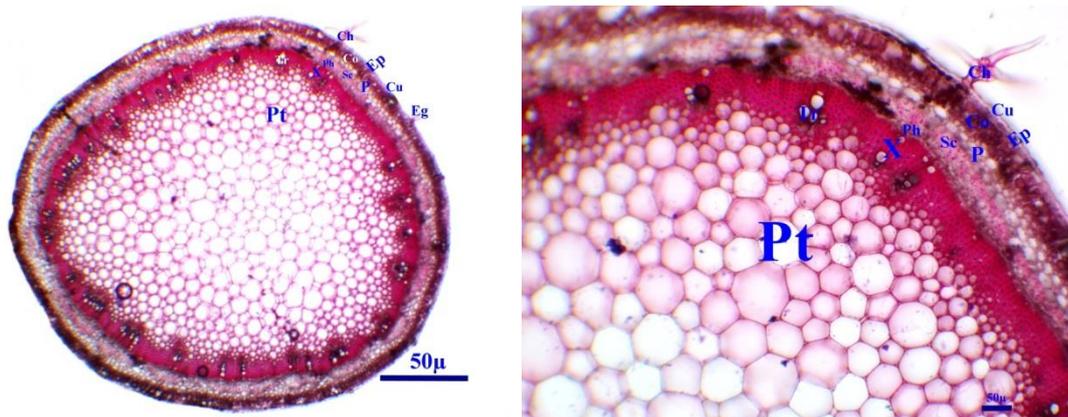


Figure 3. Cross-section of the stem of *V. racemiferum*. Eg: Eglandular hair, Ch: Compound hair, Cu: Cuticle, Ep: Epidermis, Co: Collenchyma, P: Parenchyma, Sc: Sclerenchyma, Ph: Phloem, X: Xylem, Tr: Trachea, Pt: Pith region

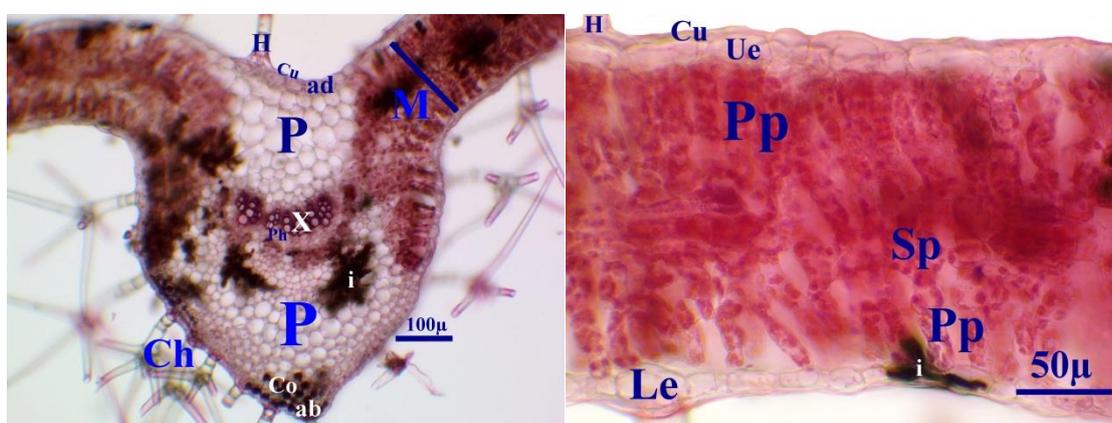


Figure 4. Cross-section of the leaves of *V. racemiferum*. H: Hair, Ch: Compound hair, M: Mesophyll layer, ad: Adaxial surface, Co: Collenchyma, P: Parenchyma, X: Xylem, Ph: Phloem, ab: Abaxial surface, Ue: Upper epidermis, Le: Lower epidermis, Pp: Palisade parenchyma, Sp: Spongy parenchyma, i: idioblast

### 3.2. Pollen morphology

The pollen dimensions of *Verbascum racemiferum* are isopolar and radially symmetric. The shape is spheroidal with a polar axis of 10.41-14.71  $\mu$  and an equatorial diameter of 10.85-14.16  $\mu$ . The pollen dimensions of *V. racemiferum* is tricolporate (20% tricolporate). The colpus length is (7.05-12.51  $\mu$ ) and width (2.04-3.70  $\mu$ ), margins distinct, regular, and ends acute. The porus is 3.12-5.47  $\mu$  in length and 2.60-4.64  $\mu$  in width. The exine thickness ranges from 0.67 to 1.20  $\mu$ . The intine thickness is 0.35-0.73  $\mu$  thin. Exine ornamentation is reticulate (Figure 5, Table 3).

### 3.3. Seed micromorphology

According to the measurements made, the dimensions vary between species from 0.53 to 0.96 mm in length and 0.23 to 0.56 mm in width. Prismatic-oblong to ovate with  $\pm$ shallow alveolate are the shapes of seeds. The seeds' acute beak. The seed is color brown. Because of the irregular polygonal and small rectangular cells, with densely and distinct vesicles, a networklike appearance is seen and the seed surface coat is longitudinally alveolate (Figure 6, Table 4).

## 4. Discussion

Analyzes of anatomy, pollen, and seeds of the rare *Verbascum racemiferum* species studied for the first time in this study make them comparable to some of the other *Verbascum* members investigated.

It was observed that *V. racemiferum* had a very large xylem area at the root and a thick cuticle layer on the stem. In addition, the same features were also noted by researchers [12, 17-20]. In some studies [12, 17], it was stated that the cambium was indeterminate in the vascular bundle in the root, and in this study, it was found that the root had similar characteristics for the species. In the leaf, cross-section analyses of the species, densely glandular, eglandular, and multicellular branched hairs were observed on the epidermal cells. Similar results have been reported for other investigated *Verbascum* species [12, 17, 19-20]. Idioblasts seen in the mesophyll tissue of the leaf can be considered a factor for identification within the genus *Verbascum* [25]. In this study, the presence of idioblasts in leaf mesophyll was determined and this feature was also noted in other studies [15, 18].

Table 3. Pollen morphological characters of *V. racemiferum*

Species	P ( $\mu$ ) min (mean) max	E ( $\mu$ ) min (mean) max	P/E ratio	Shape	Clg ( $\mu$ ) min (mean) max	Clt ( $\mu$ ) min (mean) max	Plg ( $\mu$ ) min (mean) max	Plt ( $\mu$ ) min (mean) max	Ex ( $\mu$ ) min (mean) max	In ( $\mu$ ) min (mean) max	Apt	Or
<i>V. racemiferum</i>	10.41 (11.70) 14.71	10.85 (12.12) 14.16	0.96	Spheroidal	7.05 (9.38) 12.51	2.04 (2.98) 3.70	3.12 (4.25) 5.47	2.60 (3.80) 4.64	0.67 (0.93) 1.20	0.35 (0.52) 0.73	80% Tricolporate 20% Tricolpate	Reticulate

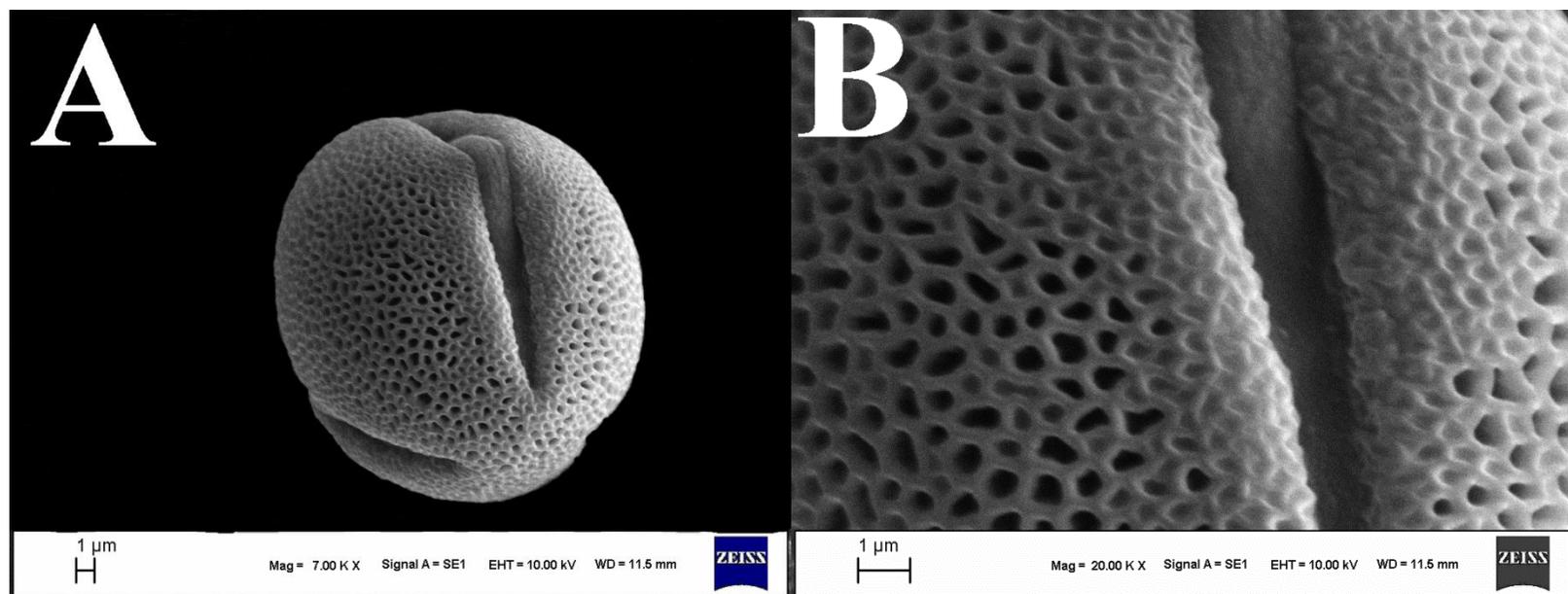
Figure 5. Scanning electron micrographs of pollen of *V. racemiferum*. A- Equatorial view, B- Exine sculpturing

Table 4. Seed morphological characters of *V. racemiferum*

Species	Group	Length (mm) min (mean) max	Width (mm) min (mean) max	Colour	Shape	Seed surface
<i>V. racemiferum</i>	F	0.53 (0.78) 0.96	0.23 (0.41) 0.57	Brown	Prismatic-oblong to ovate, with ±shallow alveolate, deep and broad back, acute beak	Irregular polygonal and small rectangular cells, with densely and distinct vesicles

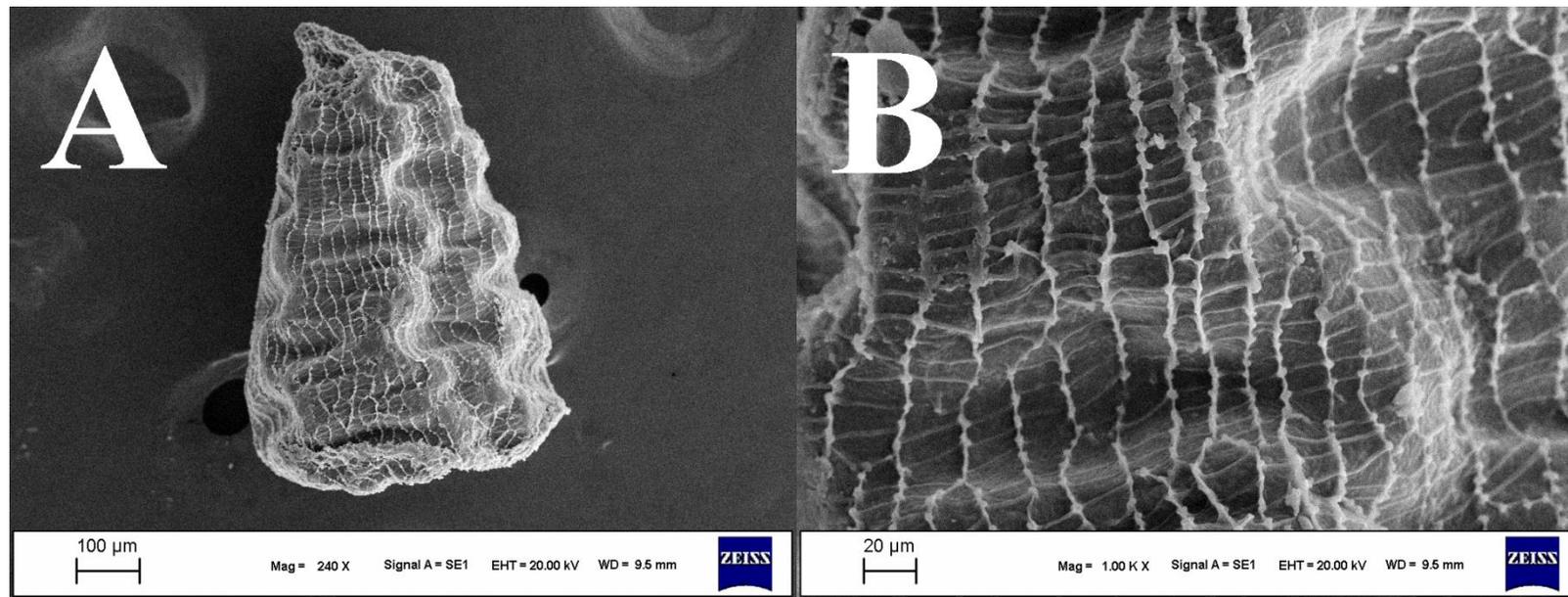


Figure 6. Scanning electron micrographs of seed of *V. racemiferum*. A- General appearance, B- Surface ornamentation

The pollen of rare *V. racemiferum* species isopolar and radially symmetric, spheroidal, tricolporate (there is tricolpate), the exine exhibits a tectate structure and reticulate ornamentation. Baser [13], in his study on *Verbascum* (8 species), established the pollen grains as tricolporate and tricolpate. Aktas et al. [12] determined the pollen grain as tricolpate in their study on the endemic *Verbascum* species. Aktas [11] noted the pollen grain as tricolpate in his study on *Verbascum* species. Öztürk et al. [10] reported that there were tricolporate and tricolpate aperture types in their pollen study on *Verbascum* (F groups). Al-Hadeethy et al. [9] in their study on 20 *Verbascum* species in Iraq, reported that the aperture types were tricolporate. In addition, it has been reported in other studies that the aperture type of *Verbascum* has a tricolporate aperture type [8, 15].

The pollen of the examined species was determined as spheroidal shape. However, Aktas et al. [12] recorded the prolate pollen shape in the endemic *Verbascum* species examined. Aktas [11] stated that the pollen shape of the species he researched has subprolate. Nevertheless, Baser [13] recorded the prolate pollen shape in the *Verbascum* (8 species) taxa in the studied. Öztürk et al. [10] stated that pollen shapes were prolate-spheroidal and oblate-spheroidal in the taxa studied. Asmat et al. [8] determined that the pollen grains of *V. thapsus* was oblate-spheroidal. Kheiri et al. [15] determined that the pollen of *V. mucronatum* species was prolate-spheroidal. In addition, Al-Hadeethy et al. [9] observed the presence of prolate-spheroidal and oblate-spheroidal pollen in *Verbascum* (20 species). Morphological pollen traits observed through LM proved not very important in their taxonomic use, but sculpting examined by SEM was found to be more significant in the classification of taxa [13].

It was determined that the exine thickness of the examined species was similar to studies [8-13] conducted on some species of *Verbascum*, therefore it was concluded that this character was very little taxonomic value [9]. However, the studied species showed a neat reticulate exine sculpture pattern.

The seeds were brown when mature. The size of the seed usually ranged between 0.53 to 0.96 mm in length and 0.23 to 0.56 mm in wide. Although Attar et al. [14] demonstrated that the size of the seed showed variations among the various populations of the same species, even among seeds in the same capsule, the experimental variation design in size was limited among the species. The measurements taken in the present study were compatible with those of the common species in the studies of Attar et al. [14] and Kheiri et al. [15]. Yet, the measurements taken in this study were larger than the mean measurements of the species common in Cabi et al. [16] study.

Seeds in shape from prismatic-oblong and ovate in the species studied, and they ended in an broad, acute beak. The seed coat was longitudinally alveolate. Usually, most seeds of the species are prismatic-oblong (Table 4, Figure 6). The result regarding seed shape is more or less consistent with the results of Baser [13], Attar et al. [14], Kheiri et al. [15], and Cabi et al. [16].

The rare species examined in this study was observed to be brown. Juan et al. [7] reported that the seeds of 10 *Verbascum* species grown in Spain were dark brown or black. Attar et al. [14] noted that the seeds of 22 *Verbascum* species grown in Iran were dark brown or black. Cabi et al. [16] also reported that the seeds of 30 *Verbascum* species grown in Turkey were dark brown or brown. Aktas [11] reported that the *Verbascum x splendidum* was dark brown. Aktas et al. [12] determined that the endemic *V. exuberans* was dark brown. However, Baser [13] determined that the seeds of eight *Verbascum* taxa grown in Turkey were dark brown or brown. According to the above findings, seed color cannot be used alone to make a delimitation between taxa.

As a result, the lack of a taxonomic and anatomical study on *V. racemiferum*, a rare species, makes the research important in terms of systematically introducing the species and genus.

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## Data availability statement

There are no additional data for this paper.

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